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STUDIES ON BIRD MALARIA IN RELATION TO THE MECHANISM OF RELAPSE

Dissertation

Submitted to the Director of the School of Hygiene and Public Health of the Johns Hopkins University in Conformity with the Requirements for the Degree of Doctor of Science in Hygiene.

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June 1923

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Studies on Bird Malaria in Relation to the Mechanism of Relapse

General Introduction

The observations and experiments reported in this paper were made for the purpose of aiding in the solution of certain fundamental problems in malaria. Under ordinary circumstances it is impossible to carry on such investigations with human patients, hence the malarial organism of birds, Proteosoma praecox, was used. This and similar avian parasites have been employed by many investigators in the past for the study of malaria and have led to results of the utmost importance that could be translated almost directly into terms of human malaria, such as the significance of exflagellation and fertilization discovered by MacCallum (1897) in the halteridium of birds and the mosquito transmission of malaria which was first worked out with the Proteosoma of birds by Ross (1898). The principle problems attacked by the writer involve (1) a statistical study of the relations between the parasitic invasion of the bird by the malarial organism and the changes in the blood picture during the course of the infection, (2) histological and pathological studies of the tissues of birds

during the infection and at various intervals after the infection has disappeared from the blood and (3) a study of the mechanism of relapse.

The writer wishes to acknowledge here her indebtedness to Dr. R. W. Hegner for his invaluable advice, constant encouragement and inspiration and to Dr. Samuel T. Darling and Dr. W. H. Taliaferro for liberal counsel and suggestions.

The Course of Infection

Introduction. The literature of bird malaria contains little or no information on the exact nature of the infection in this animal. The data available are purely qualitative and apparently have failed to indicate the precise correlation between the number of parasites and the corresponding blood pictures. This has led to conclusions that in the light of the results of the present investigation are not well founded. Danilewsky (1890) was right when he stated that the severity of the disease is in direct relationship to the number of parasites present but his was rather an assumption than a conclusion drawn from exact and precise enumerative methods. His belief that malaria in birds is characterized by a definite periodicity varying from four to five days does not seem to be borne

out by the facts even though spontaneous rises in the number of parasites have been observed on various occasions. Such rises are not periodic but are capriciously irregular and cannot be correlated with corresponding rises in the temperature as will be shown by data further on. Danilewsky described malaria in birds as an acute infection with a sharp rise in temperature. Koch (1899) and Ruge (1901) were also of the opinion that malaria in birds is of an acute nature and that after the disappearance of the parasites from the peripheral blood a complete cure and then an immunity is established.

On the other hand Wasilewsky (1904), Maldouan (1912), Whitmore (1918) and Prowazek (1920) believe that malaria is usually a chronic infection. Such an infection may last from a few weeks to a few years and if parasites are present in the body, any stage of the asexual cycle may be found in the peripheral blood.

Problem. It was thought to be of interest to obtain complete graphic representations of a relatively large number of infections and ascertain whether or not there is an analogy between such infections and those in man, and it was particularly desirable to draw a parallelism of an enumerative nature between the prevalence of the parasites

and the corresponding drama that takes place in the blood. It was therefore essential to make daily blood counts of the series of birds experimented on.

In the following part of the present work the main question that presents itself is this: Does Proteosoma praecox set up an infection that it essentially the same in all the canaries handled, or does it produce various kinds of infections? Detailed studies were made of 23 birds and the results of the experiments clearly indicate that very definite and relatively clear cut types of infections exist in Proteosoma - infected birds and those types have been classified in three groups, namely, acute primary infections, extended irregular infections and benign infections.

Material. The canary (Serinus canaria) was used as an experimental animal. Such a bird rarely shows a spontaneous infection in nature and furthermore is readily available and easily kept in captivity for relatively long periods of time. The strain of Proteosoma praecox was obtained in 1918 by the laboratory of Medical Zoology from Dr. Eugene R. Whitmore of the George Washington University. Dr. Whitmore originally obtained the strain in 1913 from sparrows in New York and carried on the infection in canaries.

Methods. Inoculations were made as follows. About 0.3 to 0.4 cc. of blood was drawn into a syringe from a vein of an infected bird's wing, diluted with 0.9 per cent of salt solution and about half a cc. of the dilution was injected into the abdominal cavity or the breast muscle or both of an uninfected bird. The parasites appear in the peripheral circulation in from 9 - 14 days as a rule but sometimes not until 24 days have elapsed.

Thomson in 1911 devised a new counting pipette for estimating the number of leucocytes and parasites per cmm. of blood. He was able to enumerate quickly and quite accurately the leucocytes and parasites simultaneously with the red cell count. In working with bird's blood the writer found it easier and more satisfactory to resort to the usual method of counting with the standard haemacytometer and the Levy counting chamber.

Blood was obtained from a small wing vein by quickly puncturing with a sharp and clean entomological needle after removal of the local feathers and thorough cleansing with alcohol. The error due to pressure or to the slow superficial circulation in the capillaries as is the case with human material is thus completely eliminated

since the blood flows freely and directly from the vein. Together with the red cell counts thin and even blood films were made for the enumeration of parasites and in some cases for the corresponding differential count. Enumerating the parasites present per ten thousand erythrocytes of each sample of blood and having the corresponding blood count of that day, it was an easy matter to calculate the number of parasites per cmm. of blood.

In order to ascertain the normal erythrocyte count a blood count was made of each bird on or before the day of injection. The normal number of red cells in fowls has been reported by various investigators to vary from 2 million to over 4 million per cmm. In canaries the normal count was found to range from 4 and a half million to a little over five million, the mean being somewhere around four and a half million.

The leucocytes were counted in a few cases. It was found impossible to make daily absolute counts for the following reasons. A large quantity of blood is required for a white blood count; blood was also necessary for the daily red blood counts and for films. The withdrawal of such a large amount of blood would cause a

considerable distortion in the blood picture and would affect the health of the bird. Differential counts which seem to be more important than absolute counts were therefore made from the thin blood films.

Wright's stain was used throughout the work for the blood films. Osmic acid vapor fixation was found very satisfactory but Wright's gives as good a stain for the study of detailed morphology as osmic acid vapor and is at the same time easier and quicker to use.

Original Observations

1. Type 1. Acute Primary Infections.

Of the 23 birds on which detailed studies were made 4 birds had acute primary infections.

(1) Bird 9. The data obtained on this bird are presented in Table 1 and Figure 1. The incubation period lasted for 23 days, that is, the parasites first appeared in the peripheral blood 23 days after the injection and rapidly increased within a day and a half to a little over 17,000 per cmm. of blood. There was a sharp decline in the number of organisms within two days and on the sixth day there were relatively few left. After that, one or two parasites could be found with some difficulty for a few days.

At the time of inoculation the blood count showed

4,270,000 r. b. c. per cmm. of blood. The number then decreased gradually until the 23d day when it was found to be 3,800,000, a loss of almost a million. On the 25th day the blood count began to increase and kept on increasing as Table 1 indicates.

At the height of the infection the bird had severe chills, showed rapid respirations, ruffled its feathers and spent the day at the bottom of the cage. On the following day there were no noticeable symptoms of sickness.

(2) Bird 19. As Table 2 and Figure 2 indicate parasites were first demonstrated on the 12th day after injection. On the 15th day they increased to 16,000 per cmm. of blood, then fell to 11,060 and during the 18th and 19th days the infection became quite acute, showing a parasite count of 28,000, whereupon the bird succumbed.

The red blood count on the day of injection was 5,200,000 per cmm. On the 7th day it was found to be 4,800,000 and continued to decline until the death of the animal, with the exception of a slight rise on the 16th day when the organisms were in smaller numbers.

The bird was very sick for eight days. It refused food, had chills now and then and kept to the bottom of the cage.

(3) Bird 10. The incubation period in this bird lasted 14 days as Table 3 and Figure 3 indicate. The parasites increased slightly in numbers during the first five days and then rapidly increased until the 22d day when they were as numerous as 34,000 per cmm. of blood. The decline was more gradual than the rise. On the 13th day after the beginning of the infection the bird died. (By the beginning of the infection is meant the first day on which the parasites are found in the blood.)

The blood count at the beginning of the experiment was 4,765,000, dropped to 2,600,000 at the height of the infection and returned to 4,200,000 before death occurred.

(4) Bird 1 showed an infection similar to that of Bird 9 and the data are therefore omitted here.

Discussion. In the birds that have infections of type 1, there is a sharp and acute rise in the number of parasites within the very short period of from 1 to 5 days. The number may rise as high as fifty-five thousand per cmm. of blood and remains stationary at that numerical value for just a few days. The fall in the number of organisms is as a rule slightly more gradual but even so it may be considered rapid. B 9 (Fig. 1) which is typical of

this type of infection shows that within one day the parasites jumped to a peak of about 18,000. On the fourth day the count was as low as 3000 per cmm. of blood and continued to fall until after a few more days it was difficult to demonstrate more than one or two parasites in a 15 minute search.

Coincident with the rise in the parasites there is a simultaneous fall in the number of erythrocytes. But even before this, an exceedingly interesting and suggestive thing happens. Before it is possible to detect the parasites in the blood films, there is a noticeable decrease in the number of erythrocytes per cmm. of blood. This occurred in every bird that has been studied. On about the fifth or sixth day there is observed a loss of about half a million red cells and this loss continues with a more or less definite acceleration. As soon as the parasites are first detected there is a sudden fall in the blood count, a fall to sometimes 2 million cells and very often even lower than that. Then, when the infection subsides rapidly and promptly there occurs a restoration in the number of the red cells and that restoration continues with a conspicuous acceleration until the number becomes quite in excess of the normal blood count. This phenomenon has been described by various authors to occur in human

post-malarial cases and also in hookworm disease. It is an indication of a hyperactivity on the part of the hematopoietic organs which were originally stimulated to excess work by the destruction of erythrocytes. In the case of birds, the marrow, which is the only blood manufacturing organ, continues to produce large numbers of red cells, even though the destruction of red cells has ceased. Such a conspicuous excess in the red cells lasted a little over six months in two birds (Bird 9 and Bird 5). This interesting reaction might also suggest that parasites present in relatively low numbers either in the internal organs or in the peripheral circulation act as a stimulant. However, this is merely a speculation.

The fact that the blood count invariably falls a number of days (5-10) before it is possible to demonstrate the parasites in the blood films strongly suggests that multiplication goes on causing a considerable and noticeable destruction of the red cells. It is quite evident that the parasites must reach a certain numerical value before they become demonstrable, no matter how careful and how patient the search might be. More efficient methods for the detection of parasites when the infection is ex-

tremely low are still to be devised. By means of Ross's method of thick blood films it is possible to get a much higher percentage of positives than by the usual method of thin films. Unfortunately this method cannot be employed in the study of bird malaria for one must bear in mind that here the red cells are nucleated. The nuclei are not dehaemoglobinized in the process and are collected in large masses which completely obscure the picture.

A mere glance at Figures 1, 2 and 3 will show that the number of parasites increased very conspicuously a day or so after they were first demonstrable in the outer circulation. Just what stimulates this reaction the writer is not ready to state but undoubtedly it is very important from the immunological standpoint. Of similar interest is the very great and sudden drop in the number of parasites a day or so after they have reached the high peak. Is this sudden decline in the number of parasites due to some protective mechanism on the part of the host or does it signify that sporulation has suddenly been arrested?

The rapid decrease in the number of parasites per cmm. of blood indicates that some condition unfavorable to the parasites has arisen in the blood of the bird. Evidence for such a statement will be brought forth in a paper that is to appear at a later date, in which a statistical analysis

of the coefficients of variation according to the method of Taliaferro and Taliaferro (1922) for differentiating the effects of resistance, seems to designate that the sharp decline in the number of the parasites can only be explained by the assumption that some factor has killed large numbers of them. These analyses show that such a phenomenon cannot be explained by the assumption of a retardation in the rate of reproduction. Even if complete inhibition of the reproduction of the parasites had occurred, the number of parasites would have remained constant, provided they were not destroyed.

In speaking of the unfavorable condition for the parasites that has arisen in the host special meaning is here attached to phagocytosis. The following table shows that with the increase in the number of organisms there is a corresponding preponderance in the number of large mononuclear leucocytes.

Bird 13

	Dec.15	Dec.22	Dec.27	Dec.28	Jan.2	Jan.3	Jan.5	Jan.8
Large mononuclear leucocytes	61%	64%	52%	52%	74%	77.5%	78%	70%
Lymphocytes	31.5%	20%	30%	32%	11%	13.5%	15%	20%
Pseudopolymorphs	4%	12%	14.5%	14.5%	12.5%	9%	5%	7%
Eosinophiles	--	--	--	--	--	.9%	--	2%
Mast cells	3.5%	4%	3.5%	1.5%	2.5%	.9%	2%	1%
Parasites per cmm.	one	4,900	--	1,908	21,105	13,345	17,835	4,230

The predominating leucocyte in the circulation of normal birds is the lymphocyte. It is found in from 50-60% of the total absolute count. The large mononuclear cells, however, are in smaller numbers in clean birds but in infected birds they are found in excess and are observed to fluctuate with the fluctuations of the parasites. In some infections simultaneous with the pyrexial peak their percentage was as high as 78.

It is only infrequently that actual phagocytosis of parasites by the mononuclear leucocytes is actually observed in the peripheral circulation. Only in one bird (B 10) (Plate III, fig. 1) was it possible to observe a marked and active phagocytosis. Stained blood smears of that bird show a considerable number of mononuclear cells to which parasites or debris of parasites are

attached. Of the twenty-three cases studied this was the only one in which such conspicuous phagocytosis was directly observed. However, this is not surprising because the destruction of protozoa by white blood cells as reported in the literature is an extremely rapid process and can only rarely be caught.

Even though actual phagocytosis is not always demonstrable in the blood, the reaction in the mononuclear count suggests that those cells might have something to do with the sudden decrease in the number of parasites. This would signify furthermore that the opsomic index has jumped up too. The writer can only go as far as to assume that such might be the case. The present belief is that phagocytosis is largely responsible for the decline in the parasite count, yet other factors may also be involved in the process.

This reaction in the mononuclear count has been described by Thomson (1912) and Darling (1909) in the case of human malaria. Their results were based on a large number of cases but even though few figures have been presented here, it is quite interesting to note an analogy between such a phenomenon in birds and that in human beings.

The question might be raised as to what evidence there is that the parasites do not simply migrate to the tissues?

If that were true the number of the parasites in the tissues would increase tremendously whenever the number in the blood decreased. The writer has failed to find such a condition. In fact the results presented in the second part of this work clearly indicate that as the parasites increase in the blood they also increase in the tissues and vice versa, when they decrease in the blood they also decrease in the tissues.

The infection in Bird 19 (Figure 2) was also of the acute primary type. In this case there was a slight variation. On the 15-17 days there was a slight fall in the parasite count and an attempt of regeneration in the red blood cells. This cannot be explained as due to experimental errors in technique since the change was noticed after two repeated examinations. It probably was due to a sporadic increase in the protective mechanism of the animal. Immediately after this the organisms reached the very high number of 20,000 per cmm. of blood. The bird then succumbed to the infection.

It was observed in this bird, and in two previous birds with acute infections of a parallel nature, that only the normoblasts were parasitized. Most of them contained

as many as 3 or 4 trophozoites. The old red cells which were in small proportion being practically free from the invasion.

This interesting picture suggests one or two things. First that sporulation at a maximum speed goes on in the bone marrow and that the freshly produced red cells are being attacked as soon as they are produced and are thence discharged into the circulation. Second, that the parasites take the route of least resistance since the normoblasts evidently have a more delicate membrane than the erythrocytes and hence are more easily penetrated.

The results of studies of bone marrow taken from birds that died or were sacrificed at that stage of the infection are compatible with the first belief. This point will be taken up further on in the studies of tissues.

2. Type 2. Extended Irregular Infections.

Sixteen birds exhibited an infection of that nature.

(1) Bird 4 (Table 4, Figure 4) showed an incubation period of 14 days. On the first day of the infection 8,000 parasites were present per cmm. The infection increased gradually and steadily until the 31st day when the count was as high as 55,000. A sudden drop then occurred within the next two days, to 5,000. As figure 3 and table 3 show, there

was a second and minor increase on the 37th day and a third but conspicuous increase on the 41st day. The infection completely subsided on the 46th day.

The normal blood count was 5,000,000. On the day the parasites first appeared in the blood it had fallen to 4,050,000 and continued to decrease to about 3,000,000 and remained in the vicinity of that figure for 9 days whereupon there occurred a rise to 4,000,000. With the subsequent two peaks in the parasite count there occurred corresponding drops in the blood count and ultimately at the end of the infection the red blood corpuscles increased to 5,250,000. The bird showed symptoms of sickness on the 30th day after the experiment was begun and remained so with varying fluctuations for about 9 days and then apparently recovered.

(2) Birds 6 and 13 showed infections of a nature parallel to that of Bird 4. Parasites were found in the blood in abundance for nearly two months and fluctuations occurred similar to those described above.

(3) Bird 3 (Fig. 5, table 5) had an infection of a severity less than that in Bird 4. The incubation period was relatively shorter, lasting 12 days instead of 14. Here there occurred a primary rise to 15,000 organisms within one day,

followed by a drop to 6,000 and a subsequent increase to 31,000. Slight fluctuations lasted for 7 days following this secondary rise and then the infection promptly multiplied itself to 52,000 parasites per cmm. whereupon the bird succumbed.

Six days after the experiment was begun the blood count showed a decrease from 5,000,000 to 4,450,000 red cells per cmm. This continued to decrease to 2,900,000 when there was a corresponding rise in the numbers of parasites. Fluctuations in the blood count lasted for 17 days until the death of the animal as Figure 4 and table 4 indicate.

The bird was very sick for 4 days, and the infection then proved to be fatal.

(4) Bird 7 had an infection of the same character as bird 3 with the exceptions that the primary rise was somewhat higher, i.e., to 19,000 parasites and the last peak was considerably lower, that is, the largest number of parasites found was 32,000 per cmm. The bird survived the infection and showed no relapses.

(5) Bird 17 as figure 6 and table 6 indicate, had a still milder infection than the first two typical infections that have been described above. The parasites were first seen

on the 14th day after injection. There was a mild primary rise to approximately 6,000, followed by a decline to almost zero and a subsequent increase to 21,000. Within 8 days after this rise the infection had almost subsided and 21 days after the beginning of the infection no organisms were to be found. Five days later the bird had a relatively severe relapse which lasted for two days.

The normal blood count was 4,750,000 and a noticeable decrease was seen on the 8th day after the injection. On the 14th day the count was very close to 4,000,000 which kept decreasing to 2,500,000 and then gradually increased to an excess of a little over half million of the original blood count.

No severe symptoms of sickness were noticed at any stage of the infection.

(6) Birds 18 and 21 had infections lasting for about 23 days and showing fluctuations in the blood count and parasitic count of a similar nature to the one described above.

(7) Birds 14, as Figure 7 and Table 7 show, had an incubation period of 12 days and the infection, even though mild, lasted for 19 days. On the 12th day after injection, 2,064 parasites were demonstrated in the blood. There was a rapid increase to 10,500 within two days and then a drop to

4,100 which was followed by a secondary rise to 8,190 organisms. The infection subsided completely 7 days later. For a period of 31 days no parasites were detectable on the daily examinations of smears but on the 31st day parasites reappeared for 3 days.

There was a tremendous anaemia in spite of the fact that the infection was relatively mild. The normal blood count was 5,200,000 per cmm. and subsequently fell to 2,160,000. Just before the relapse the red cells had risen again to 5,200,000 and then fell again at the time of the relapse (Fig. 9).

The bird was very sick on the 4th day of the infection, that is, on the 4th day after the parasites first appeared in the blood. It shortly recovered and did not show any more symptoms for the remaining days of the infection.

(8) With the exception of very slight variations other birds had irregular extended infections similar to that in Bird 14. The parasites were demonstrable in the blood for a period of a month. They never increased to over 11,000 per cmm. and showed fluctuations of the same character. Extreme anaemia occurred in all of them, but such anaemia only lasted for a couple of days and regenera-

tion in the blood occurred with the same promptitude and regularity as described above.

Those birds bore the following numbers: 2, 11, 12, 16, 23, 22, 8.

Discussion. To this group belongs the greatest percentage of infections. They are characterized by a relatively long duration and by fluctuations of various degrees. In these infections just as in those belonging to the first group a drop invariably occurs in the blood count before the infection becomes apparent and then an increase to excess in the red blood count after the infection has subsided. Having already discussed these phenomena it will not be necessary to consider them again.

A mere glance at Figures 3, 4, 14, and 17 will clearly show that tremendous fluctuations and ranges in the severity of the malady exist in the extended irregular type of infection. In the literature reference is made to chronic infections in bird-malaria. One usually thinks of a chronic condition as one of a very slow, relatively mild, and extended duration. It does not seem quite plausible, however, to call the extended infections in birds "chronic" since at times they become exceedingly aggravated and acute.

In most infections of this group the initial rise

is more gradual and covers a longer period of days. The falls, however, may be, and usually are, just as sudden and as great as those of acute infections. Coincident with the fluctuations in the numbers of parasites occur similar changes in the blood picture. Instead of having a prompt fall followed by a rise in the blood as is the case in the acute infection here there are a number of rises and falls, some more conspicuous than the others.

Bird 4 (Figure 4) had a very severe infection at the height of which 55,000 organisms were counted per cmm. of blood but the loss in the red blood cells was not so serious as one might have expected. The blood picture shows that throughout the malady no severe anaemia occurred. This is quite striking and would indicate that in spite of the repeated increases in the number of parasites the host resisted the infection and severe injuries did not develop. Another interesting thing in this and the similar cases is that the animal was practically altogether refractory to symptoms of illness.

At each peak is found a large percentage of trophozoites and segmenters. What the aetiology of such an occurrence is the writer cannot state, but one thing is quite

clear and that is that such sporulation has no regularity whatsoever. In the statistical analyses of the infections which will be published at a future date there is no indication of a periodicity in sporulation.

In Bird 3 (Figure 5) the fluctuation was even greater than in Bird 4. Four rises occurred and at the 4th one the bird died. We undoubtedly have to do here with a difference in the resistance on the part of the host. Unlike Bird 4, Bird 3 suffered from a considerable anaemia (3 million per cmm.) and even though attempts of regeneration of red cells were noticeable, the protective mechanism of the bird was a failure.

In Bird 14 on the other hand, the infection was much milder, the highest peak representing a value of a little over 10,000 per cmm. of blood, but despite this the destruction of red blood cells was considerable. For two days the blood count was as low as 2 million and yet there were relatively few parasites in the blood. The only explanation that can be offered for such a phenomenon is that probably the infection is in excess in the bone marrow over that in the peripheral circulation setting up a condition there which hampers or delays due regeneration of the red blood cells.

3. Type 3. Benign Infections.

(1) Bird 15 (Table 8 and Figure 8) showed parasites on the 13th day after injection. Within one day the parasites increased from 1,276 to 4,200 per cmm. of blood and three days later none are demonstrable.

The blood count decreased from 4,950,000 to 4,000,000 and then gradually rose to normal and supernormal.

The bird was perfectly well throughout the four days of infection.

(2) Bird 20 (Table 9 and Figure 9) had an infection of a milder nature as far as the number of organisms is concerned but the malady was much more severe and of a longer duration than the above case. Parasites were first seen on the 9th day and then within the following nine days gradually increased from 1,804 to 3,000 per cmm. of blood. On the 18th day of the infection parasites were no more visible in the blood smears.

The destruction of blood in this bird was considerable, however. From 5,500,000 it dropped to 2,500,000 just a few days after the infection began. There occurred due regeneration, however, within the following days.

The bird was quite sick on the second day of the infection and continued to show symptoms of illness for five days.

(3) Bird 5 showed organisms on the 10th day after infection and on the following day there were none to be found. Continuous blood examinations were made but no parasites were detectable.

The blood showed a very insignificant reduction in red blood cells and the bird was apparently well.

Discussion. A glance at Figures 6 and 7 leads to the conclusion that these infections were exceedingly mild. The general nature of such infections reminds one of the similar conditions in some human malarial cases. Such people are able to go about their daily work and are not subject to any discomfort and hence are known as "ambulatory cases."

Just why birds, who receive the usual dose of infected blood, do not develop fairly severe infections it is impossible to state. Again it is necessary to take the protective mechanism of the host into consideration. It would appear that partial immunity is responsible for such mild infections. Five birds did not develop the disease at all showing that they were completely and adequately protected. The whole matter seems to be an interesting immunological problem.

4. General Observations.

(1) Stages in the life cycle of parasites found in the blood. In all the various types of infection, from

the early days of the infection all stages of development in the life cycle of Proteosoma can be seen in the blood. However, one stage predominates at all times during the course of the infection. At the very beginning, although trophozoites and gametocytes are found, the predominating stage is the well formed schizont. At the height of the infection and by that is meant any one of the peaks, whether it be the acute or extended type, the percentage of young trophozoites is the highest, varying from 70 - 85. When the infection is on the decline and verge of subsiding one mostly finds gametocytes but also schizonts in fairly large numbers. The fact that gametocytes in human malaria are more resistant than the other stages of the parasite probably holds true in bird malaria also.

(2) Variations in the incubation period. As the data which have been presented indicate, the incubation period is not of the same duration in all of the birds. An occurrence of that nature suggests two causes, firstly, that the doses of infected blood injected, contained varying numbers of organisms, and secondly, that some birds display a greater degree of immunity than others. The writer is inclined to believe that the latter factor is the more important of the two. Some birds that were injected with sparsely parasitized

blood developed a most severe infection. On the other hand, other birds received heavily infected blood and developed a very mild infection or none at all. All this would indicate that the host either resists the multiplication of parasites shortly after injection or fails to do so, thus causing a much shorter incubation period. Dr. Whitmore believes that the stage of the parasite inoculated has something to do with the length of incubation. When segmenting parasites free from red cells are injected they can be easily attacked by phagocytes while on the other hand well formed intracellular organisms have a better chance to survive.

(3) Fluctuation in temperature. Temperature readings were made on a number of birds as often as 15 times a day. In order to get a fair average five readings were made at short intervals of a few minutes and this was repeated three times. It was found that no curves of any significance whatsoever could be shown from the readings. Fluctuations were great within a minute or so indicating that the bird is of a highly sensitive and emotional nature. For example, the temperature readings of B 4 on May 5 are as follows: 9.30 A. M., 105.1° F.; 9.32, 108.2° F.; 9.34, 104.4° F.; 1.00 P. M., 108° F.; 1.02, 106.2° F.; 1.06, 107.3° F.; 4.00, 104.2° F.; 4.02, 108.3° F.;

4.05, 106° F. These readings were taken before the parasites appeared in the blood and several days later when the infection was quite active the same kind of variations were obtained.

The Sergent brothers (1918) obtained the same fluctuations and conclude that the temperature has no relationship with the course of the infection.

(4) Changes in the Blood. The most conspicuous changes in the blood picture besides anaemia and the mononuclear reaction already described, are (1) a great increase in the normoblasts which varies according to the severity of the disease, (2) endoglobular degeneration of erythrocytes, and (3) poïkilocytosis of varied degrees.

(The summary will be found on page 56).

Part II. Histological and Pathological Studies of Infected Birds

Introduction. As early as 1889 Danilewsky in his "Parasitologie Comparee du Sang" writes of the changes in the haematopoitic organs of birds infected with haemocytozoa and describes the macrophages which contain considerable quantities of pigment and debris of parasites. Kruse in the same year speaks of the enlarged and blackened spleen in infected birds and of the pigment enclosed in large cells in the capillaries of the spleen, liver, lung, and bone-marrow.

Labbe in 1884 draws attention in a general way to the gross changes in the organs of infected birds and MacCallum in 1898 working on a large number of infected birds describes in great detail the anatomo-pathological changes in the organisms of birds infected with Halteridium.

No data are available in the literature regarding the conditions of and findings in organs between relapses when there is no evidence of blood infection.

Problem. Investigations have been undertaken to determine (1) the specific location of parasites during the peripheral infection and at varying apyretic intervals between relapses, (2) the stages of the life cycle of the or-

ganisms during these periods and (3) the condition of the internal organs.

Since it was found during the course of the work that the spleen and bone marrow exhibit the most interesting and most important changes, the other organs will only be mentioned briefly.

Material. The material was obtained from birds that were sacrificed at desired points of the infection and at varying stages of the apyrexial period, and from birds that died accidentally or fell victims to the malady.

Tissue smears of the spleen, bone-marrow, liver, kidney and brain were made at autopsy and material was also fixed for the purpose of making sections.

Methods. Various fixative agents were given a trial such as Zenker's, Bouin's, and Schaudinn's solutions. Zenker's was found to be the most satisfactory for this particular work and hence was resorted to.

Among the stains tried out were the following, Wolbach's modification of Giemsa, Iron Haematoxylin, Mann's stain and Phosphotungstic. Giemsa and Phosphotungstic stains gave good results and were both used, but the former was used more frequently and more liberally.

Technique for smear preparation. A small bit of the tissue was thoroughly teased out in a tiny drop of saline and of this mixture smears were made upon slides. The preparations were dried in the air and fixed in absolute methyl alcohol for 5 minutes after which the excess alcohol was removed with filter paper. The slides were then flooded with freshly diluted Giemsa stain for one hour and then washed in a stream of water. The stain was prepared according to directions given in Mallory and Wright's "Pathological Technique."

Histological Technique for Sections. Small sections of about 2 mm. were fixed in Zenker's fluid saturated with corrosive sublimate. The material was dehydrated in the graded alcohols and in xylol, embedded, and sections were then cut 3 microns thick. The slides were treated in the usual way first in xylol, then followed by the graded alcohols. The extra steps taken were as follows: (1) In order to remove the corrosive sublimate crystals deposited in the tissues the sections were treated with Lugol's solution and then washed in 95% alcohol to remove the yellow color. (2) After rinsing in distilled water the sections were placed for 10 minutes in a 0.5 per cent. solution of sodium hyposulphite to remove the iodin then thoroughly washed in running water

followed by distilled water. The slides were then stained in a freshly diluted Giemsa solution from 12-24 hours. After the first hour the staining mixture was poured off and replaced by a fresh solution.

Differentiation was carried out by means of acetone and xylol. The slides were finally cleared in xylol and mounted in balsam.

The formula used for the stain was as follows:

Azur II eosin	0.3 gm.
Azur II	0.8 gm.
Glycerin (Merck's)	125 c.c.
Methyl alcohol "	375 c.c.

A dilution of 1 drop to 1 c.c. of slightly alkaline water was employed.

Original Investigation.

1. General. As has been stated above detailed pathological studies will only be given of the spleen and bone marrow owing to the fact that important changes were found only in these organs. For the sake of comparison as well as for a better understanding of what follows, it seems desirable at this point to discuss the normal anatomy and histology of the spleen and bone marrow of birds as given by previous investigators.

Spleen. Mühler (1805), Whiting (1896) and MacCallum (1898) have given detailed accounts of the structure of the spleen of birds. It has been noticed by them that the capsule in smaller birds possesses no trabecular system; the vessels, however, are surrounded by a certain amount of connective tissue. It is near these masses of connective tissue that the pigment is especially deposited. At the points where the arterioles branch off, bodies of lymphoid tissue are seen and they are separated from the connective tissue by a double row of elongated cells which are the endothelial cells.

In sections of normal birds the above mentioned authors noted that the pulp proper is made up of large cells and runs in bands. These pulp bands are made up of wide capillary spaces which anastamose freely in a net-work of large pulp cells with characteristic nuclei that are either round or oval or polyhedral. These cells are most closely aggregated at the margins of the bands of pulp and in infected birds are found to be highly phagocytic. They form the boundaries of the blood spaces and are essentially endothelial in nature.

Bone-marrow. In 1891 Bizzozzero, an Italian investigator, pointed out for the first time that unlike the mammalian bone marrow, in birds the venous capillaries are lined by a very delicate nucleated membrane. He and his

collaborator Torre for the first time in 1880 described the various kinds of leucocytes seen in the marrow and general circulation of birds. They found that the venous capillaries have a richer supply of leucocytes than the other vessels. The type of leucocyte that predominates is the eosinophile. The disposition of the various elements within the vascular lumen is very definite. The fully developed red blood corpuscles are in the central area while the erythroblasts and leucocytes occupy the periphery of the vessel. This has a direct bearing on the haematopoitic function of the bone marrow, showing that the manufacturing of blood corpuscles goes on at the periphery of the venous capillaries and not in the larger vessels. Besides the vascular network of the bone-marrow, a part that is less important in its function but just as conspicuous histologically, is the parenchyma. It has a variable quantity of adipose tissue and leucocytes. The fat content varies greatly in the various bones and in various birds. As to the leucocytes, one sees the small mononuclear, the large mononuclear and a predomination of eosinophiles with bright granules or rodlets. There is a very intimate connection between the parenchyma and the content of the vessels owing to the fine membrane of the capillaries.

2. Conditions found in the tissues of birds at a maximum infection. Two birds were sacrificed at this stage for the purpose of study and a third bird that was used died as a result of the malady.

Spleen. In normal birds the spleen is of a bright red color and measures about 3 mm. x 2 mm. On the other hand, the infected birds showed at autopsy a black spleen of a maximum size of 15 mm. x 5 mm.

Tissue sections.

In highly parasitized birds it was observed that the endothelial cells which form the boundaries of the blood spaces are considerably distended, showing large clumps of pigment and smaller granules of pigment of a much brighter color. Not only do these cells contain the by products of the parasites and destroyed haemoglobin but also debris of red blood corpuscles and apparently phagocytized organisms.

In the smaller capillaries many parasitized red blood cells are seen to be crowded in the lumen of the vessels thus causing them to appear much more voluminous than the normal capillaries. Large clumps of pigment are conspicuously and widely distributed in the spaces between the splenic bands. Such clumps have no particular shape nor form.

Macrophages are seen in large numbers and practically all are engorged with pigment. Two distinct varieties of pig-

ment are observed just as is the case in human malaria, i.e., the dark brown pigment, which is the product of the metabolic process of the parasites, and the light brown pigment which is produced when the haemoglobin is set free at the destruction of the eryghrocytes.

An interesting picture that was uniformally observed in birds autopsied at the height of the infection was the occurrence of a large number of mononuclear cells. They have deeply staining nuclei and are seen within the sinuses. Such an increase in the nononuclear cells has been described by Morris (1907) in anaemic rabbits. Not only is such a condition to be seen in the spleen but is also a conspicuous picture in the blood as has already been described above. These cells are apparently of a phagocytic nature since it was observed that many of them contain fragments of red blood corpuscles, pigment and degenerated parasites.

Ovoid or round cells with spindle shaped nuclei which stain very brightly with Giemsa are most numerous in the sections. They are the mitotic figures of red blood cells in the various developmental stages. Their great number indicates the return of the haematopoeitic organs to embryonic conditions due to the excess destruction of the erythrocytes by the parasites.

Besides the above described elements that were found in the spleen of sick birds, numerous round or ovoid small bodies were observed either free in the splenic pulp or apparently attached to mononuclear cells. These bodies which have a very definite membrane and are made up of faintly colored material, are of a refractive nature and contain one or two larger pigment granules and a few smaller granules. They have been carefully studied after various staining methods yet always exhibit more or less the same characteristics. They are undoubtedly too small to be macrophages and are believed to be young extracellular organisms. The writer was not at first convinced that these bodies were parasites due to the fact that they were observed in the pulp, extracellular, or attached to mononuclear cells. But a study of smears of the same tissue strongly corroborated the belief and led to an ultimate conclusion that these small pigmented bodies are young parasites.

Smears of spleen tissue- While for pathological studies, tissue sections are indispensable the writer is firmly convinced that morphological studies of the organisms should be basedupon results from tissue smears. In smears, one instead of three dimensions are involved and henceeach individual cell stains out remarkably clear and the parasites assume the proper staining

colors and hence cannot be mistaken for anything else.

In the smears not only are young and old parasitized blood corpuscles present, but numerous organisms are seen free. Mature shizonts have never been observed to be extracellular while trophozoites, merozoites and a few gametocytes have invariably been observed either entirely free or attached to mononuclear cells (Plate I, figs. 1 and 4).

Small clumps of pigment were observed in the smear but not as much as in the sections.

Bone marrow. The bone marrow was obtained from the tibia and febula as well as from the larger wing bones.

In normal birds the marrow is of a bright pinkish yellow or grayish pink color while infected birds exhibit a bone marrow of a grayish red color and frequently when the infection is very severe a brownish red hue. Bizzozero (1880) Korn (1881), and Denys (1887) report similar gross changes in birds that were bled experimentally. The disappearance of the normal gray is due to the atrophy of the fat particles.

Microscopic examination of sections of such material prove that there is a considerable decrease in the adipose tissue and the mitotic figures of leucocytes and erythrocytes

most numerous. On the other hand a notable dilatation occurs in the venous capillaries. The accumulation of erythroblasts as well as leucocytes is so extensive that the lumen of these vessels is practically occluded. Adult corpuscles are very few in number and most of the normoblasts are parasitized, many showing a double and triple infection. Mitotic figures in all stages of development are seen and their chromatin takes on a bright pink color when stained with Giemsa. The sections invariably reveal a marked hyperplastic condition. Non-granular mononuclear cells which are probably mycloblasts are most numerous and to many of these cells little pigmented bodies are attached just as in the case of infected spleens. Whether these parasites are in the process of being phagocyted or are en route to attack other red cells it is extremely difficult to determine.

In between the non granular mononuclear cells macrophages containing pigment and debris of parasites were observed.

It is noted that in birds in which great destruction of red blood corpuscles occurs important pathological changes alter the normal histology of the marrow. In the first place, there is a great dilatation of the capillaries at the expense of the parenchyma. This is due to the fact that a marked hyper-

plasia of cells occurs in the blood spaces, secondly there is a considerable atrophy of the fat cellules and thirdly mitotic stages of division are to be seen in preponderating numbers along the periphery of the venous blood spaces.

Smears. - The marrow smears indicate that sporulation is allowed to go on undisturbed. A mere glance at plate I and figures 2 and 3 clearly show that multiplication of parasites goes on in the bone marrow. Segmenters having broken off from the red blood cells are present in large numbers. Trophozoites and extremely young schizonts preponderate, either free or attached to large mononuclear cells. Only few mature erythrocytes are found and such cells are invariably free from parasites. On the other hand the normoblasts are practically all infested with two or three organisms. This finding has already been noted in the first part of the work in the case of the blood of such infected birds. Since such is the state of affairs in the blood of heavily infected birds it is believed that a great part of sporulation actually takes place in the bone marrow and that the normoblasts are attacked in the marrow and thence discharged into the outer circulation.

Liver. A considerable number of pigmented macrophages were seen in the liver sections as well as many pigmented endothelial cells. There was no marked alteration in the tissue

tissue as was observed in the spleen and marrow yet distention of the capillaries was found in various parts. Only few parasites could be detected and they were intracellular. Clumps of pigment in the sinuses and in between the pulp cells were widely distributed.

Tissue smears from the liver showed some pigment and a number of parasitized red blood cells as well as some free trophozoites and segmenters.

Kidney. The kidney in birds is essentially the same as that ofhigher animals. There was no evidence for any alteration in the histology of the tissues. Pigment was found in considerable amount and a number of parasitized red blood cells were observed.

The smears revealed a number of organisms.

Brain. No noticeable changes were found except the presence of pigment and a few parasitized red cells here and there in the sinuses. No hyperplasia was noticed.

3. Conditions Found in the Tissues of Birds at the End of Infection.

Two birds were sacrificed at the time when parasites had practically all disappeared from the blood.

The spleen was slightly enlarged and somewhat darker than the normal.

Sections of such spleens show that the endothelial cells which form the boundaries of the blood spaces have practically returned to their normal status and most of them are free from pigment. The lumen of the vessels have resumed a size very near to the normal for the red and white corpuscles are not as numerous as before. There still seems to be a hyperplasia of the mononuclear cells but not as considerable as in the highly infected birds. Parasites are found in only small numbers and no free organisms were seen in the sections.

In the tissue smears parasites were found too in practically every stage of development.

A remarkable regeneration was also observed in the bone marrow of these birds. There was a great loss of the bright red color and considerable restoration of the grayish hue. Sections of the bone marrow confirmed this outer appearance in that the adipose tissue resumed a more or less normal morphology. Mitotic figures were no more conspicuous, in fact were quite scarce and the erythroblasts and leucocytes showed a normal distribution within the lumen of the vessels. Whereas in the first cases described the normoblasts were in preponderance here the young red cells were found in small proportion. Yet granules of pigment were seen in various parts of the sections both in the macrophages -

lying free in little clumps. Quite a few parasitized red blood cells were observed, also some free parasites.

On studying the smears of the bone marrow practically all stages of proteosoma were found indicating that the asexual cycle is still in operation.

4. Conditions found in the tissues of birds during the latent infection.

a. One bird was sacrificed two weeks after the infection disappeared from the blood. Blood examinations were made during that period and with a careful search it was not possible to detect any organisms.

The spleen and bone marrow were found absolutely normal. No alterations of any kind were found in the histology of those tissues but pigment was still sparsely distributed. Lengthy and exceedingly laborious examinations of the smears of the spleens and marrow revealed a number of organisms that were perfectly normal morphologically. A few schizonts, one trophozoite, two gametocytes and some segmenters were found as seen in Plate I, figs. 5 and 6, and Plate II, figures 1, 2, 3 and 4.

b. One bird was sacrificed 31 days after the parasites had disappeared from the blood. The bird had been infected for over two weeks and after the decline of the infection completely

recuperated and blood smears did not show any organisms for 31 days.

Sections of the spleen and bone marrow indicated that complete regeneration had taken place. Three hours were spent on a number of sections and smears made from the spleen and marrow and ultimately two organisms were detected, one of which was a perfectly normal looking and beautiful segmenter and the other, an atypical schizont, (Plate II, figs. 5 and 6).

Another bird was sacrificed one month after the original infection had subsided (B 19). It was only after many hours of constant search that it was possible to demonstrate a single parasite. This young schizont is seen in Plate III, fig. 2.

c. B 9 as table 10 shows had several relapses but the original infection subsided on June 29, and its day of autopsy is counted from that date, that is, the bird was sacrificed six months after the acute infection had subsided. In this bird, too, it was possible to demonstrate one organism after the repeated examination of a large number of slides. As Plate III, fig. 5 indicates the organism is a typical young segmenter.

B 14 was autopsied 2 and a half months after the

infection. One extremely young schizont was detected with extreme difficulty. Plate III, fig. 4 .

Discussion.

In the study of tissues which has been presented above it was seen that severe changes take place in the bone marrow and spleen of heavily infected birds. Some of these findings, such as the heavily pigmented endothelial cells, and the occluded sinuses are in harmony with those reported by MacCallum, in case of Halteridium infected birds. The tremendous reduction in the adipose tissue of the bone marrow and the hyperplastic condition in the blood elements have been observed by Bizzozero and Torre in anaemic birds.

Extracellular parasites or parasites apparently attached to mononuclear cells have not previously been described.

It is tremendously interesting to note with what rapidity the regeneration and practical restoration to the normal takes place in the organs of birds. The building up processes are at a maximum speed for whereas great histological disturbances are invariably seen during the severe stages of the malady, after the apparent disappearance of the organisms from the blood, the ill effects

are conspicuously reduced. Inasmuch as the subsequent birds examined showed even greater proximity to normality it must be said that recovery in the tissues is prompt and regular. However, the fact that a few parasites remain in the tissues and can be demonstrated there in various stages of development and exhibit a normal morphology indicates that the host has not completely gotten rid of the infection and that the asexual cycle continues to some extent. It might be that the host has built up a resistance against their reproduction. Assuming that such is the condition it can only be of a temporary nature since in most birds one, two and sometimes three relapses occur at varying intervals.

The problem of actual destruction of the parasites has been of great interest in this study. As it has already been noted above, actual phagocytosis in the blood has been observed in one case; Plate III, fig 1, clearly picture such a state of affairs. Many parasites are in the process of destruction and some show marked swelling and complete degeneration. The mononuclear cells undoubtedly have a harmful effect on the parasites whether that effect is due to direct phagocytosis or to some antibody produced in the blood it is not possible to state.

In the tissues phagocytosis is seen again. Here

it is possible to observe it much more frequently than in the blood. Several large mononuclear cells have been noted to contain within their bodies pigment and debris of parasites indicating that they not only embrace the organisms but also engulf them and subsequently completely destroy them (See Plate III, fig. 3)

In studies concerning fixed tissue phagocytosis Kyes (1922) has shown that the physiological destruction of erythrocytes is accomplished by endothelial cells in the spleen. He has shown that these cells display great activity in removing bacteria from the blood stream. There is but little doubt in the writer's mind that such a phenomenon goes on in the blood and tissues of Proteosoma infected birds. It would therefore seem probable that the great reduction in the number of parasites is due to their actual destruction by fixed tissue cells as well as by the circulating phagocytes. In other words it seems as though phagocytosis goes on both in the tissues, particularly in the spleen and marrow, and in the circulating blood.

(Summary will be found on page 57)

Part III: Relapse.

Introduction. Relapse has been defined as follows: "A return of the fever and parasites after the former has ceased and the latter have disappeared from the peripheral blood; reinfection being excluded." (James, 1911).

It is an established fact that a considerable proportion of the malaria rate is due to relapse. In certain places such as Greece and Italy, in winter when the possibility of reinfection is either very slight or absent there is none the less a very considerable amount of the disease. Such an occurrence indicates that when large numbers of people are infected in a locality a certain number, after the clinical symptoms have subsided, will still harbor the parasites until conditions, concerning which little is known, bring about another attack.

It has been shown that in some places the increase in the amount of malaria at the beginning of the wet season is out of proportion to the increase in the amount of Anophelines. Before the mosquitoes can transmit the infection, it takes a certain length of time for them to breed and become infected, but the malaria rate rises

promptly after the first heavy rains even in places where the mosquito rate shows no perceptible increase.

It is therefore very evident that the etiological factor of relapse must be looked for in man himself.

Original Observations. In the work with malaria-infected canaries it was observed that a number of the birds relapsed at varying intervals, in spite of the fact that the surrounding conditions had not altered in any perceptible degree. Some birds were induced artificially to relapse either by radiation with ultra-violet rays or by injection with adrenalin.

The following data briefly outline a few of the relapsing cases.

Table 10

Bird 9./The acute infection was over on July 3d. The bird was examined until the beginning of October. On the 12th day of that month one gametocyte was found after 20 minutes search. On October 19 two parasites were found. No parasites were then detected until November 9 when the bird was radiated at a distance of 60 inches and a relapse occurred within two days when four schizonts were found. The following day one mature and one extremely young schizont were detected. From that day up to the 26th of November

no organisms were found in the peripheral blood. The bird was then inoculated with adrenalin (provocative dose = 0.00004 grams), three hours after which one schizont and one trophozoite were observed. On Dec. 29th one gameto and one schizont were found, and on the following day only one schizont was detected after a prolonged examination. From that date up to February 19th no organisms were found in the blood. The bird was then sacrificed.

Table 10

Bird 14./ The bird recovered from its infection on November 18 and on daily examinations of its blood showed no organisms until December 19, on which day 3,210 parasites were found per cmm. of blood. The parasites found were all schizonts. Microscopical examinations of the blood revealed parasites for three days and subsequent examinations were negative. On December 29, at 11 A. M. the bird received a dose of adrenalin and within two hours there were counted 2,565 parasites per cmm. of blood. The same number of parasites was present on the following day with the exception that a trophozoite was found besides the schizonts and gametocytes that were present on the previous day. After that day it was no possible to detect any organisms until January 5 when the bird received a second injection of adrenalin, but

relapse occurred. The bird was sacrificed on February 6 for tissue examination.

Bird 20. The last parasites of the peripheral infection were observed on December 20th. The blood was negative on December 21st and 22d when the bird was injected with the usual dose of adrenalin. Three hours after the injection there were found 1,590 parasites per cmm. of blood. The organisms were all schizonts. On December 24th one schizont was found and on December 25th one gametocyte. Blood examinations were negative after that.

Bird 17. The infection of Bird 17 was still active on January 5th; i. e. there were 13,920 organisms per cmm. of blood, however an injection of adrenalin was given to it in order to study the reaction. Within 4 hours the parasite count had risen to 17, 835 per cmm. of blood, but on the following day it fell to 8,265 and then completely subsided within the next two days. The bird was sacrificed on February 19th.

Bird 15. The infection of Bird 15 subsided on December 18. On the 29th a provocative dose of adrenalin was given to it, but no relapse occurred.

Discussion. The data just presented clearly indicate that

artificial relapse can be induced by means of radiation or by the injection of provocative doses of adrenalin. Since the physiological reaction of adrenalin is solely directed on the spleen it must be borne in mind that the organisms that reappear in the circulation are derived, primarily from the spleen rather than from the bone marrow. The fact that parasites can be brought out into the peripheral circulation after relatively long periods of apparent cures strengthens the finding described in the second part of this paper, i. e. that some parasites remain indefinitely in the bone marrow and spleen. The belief that they are going through a normal asexual cycle is strengthened by the fact that various stages of their developmental cycle have been found. As it has already been shown above, asexual organisms of a perfectly normal morphology were demonstrated in the spleen and bone marrow, 2 weeks, 4 weeks, 5 weeks, two and a half months and six months after the actual infections had completely disappeared from the blood. This indicates that comparatively small numbers of the asexual forms survive and that such numbers suffice to keep the infection alive for varying periods of time.

The fact that parasites are not usually demonstrable

in the blood during the apyretic interval between relapses by no means indicates that they are not there. It is only when they have attained a certain numerical value that one can find them in the blood smear. In 1910 Thomson and Ross published their results wherein mathematical methods were used to count accurately the degree of infection. By use of the thick film they were able to show the parasites between relapses whereas the usual method would not reveal the organisms. In the present work it often happened that slides that were put away as negative, upon a careful and patient reexamination proved to contain one or more parasites.

It was shown in the first and second parts of this paper that phagocytosis is probably the most important factor in the disappearance of the parasites from the outer circulation. In the acute human infections which pass into a long chronic stage in which no parasites can be found in the blood, it seems probable that the destructive process is carried to such an extent thatthe parasites are too few in the blood to find by direct microscopical examination. According to this relapse probably is brought about by the removal of the destructive agent so that the parasites are permitted to accumulate in the blood.

For the sake of clarity it seems logical here to state the various hypotheses that have been brought forth as

the causes of relapse. According to James they fall under two heads:

(1) "Some particular or peculiar form of the parasite is developed during the asexual cycle and this form is resistant to quinine and to the protective forces of the body. It remains latent until called into activity by certain forces whose action is not as yet clearly understood, when it breaks up into merozoite-like parasites which take up anew the asexual cycle.

"This is the hypothesis which explains relapses as due either to parthenogenesis of the female gamete as advanced by Canalis, Grassi, Mauer, Mab and others and worked out in detail by Schaudinn, or as due to segmentation or a parasite formed by conjugation of the young asexual forms which is the view taken by Craig."

(2) "After a diminution in their vitality due either to quinine or to the natural protective forces of the body, the asexual parasites continue to go through their normal life cycle but in a very small number relatively speaking until the causes that bring about relapse effect a renewal of vitality so that the parasites increase in numbers until the clinical symptoms of relapse are manifested.

This hypothesis has the support of Ross, Thomson, James and is slightly modified by Dignami."

In the light of the results obtained in this experimental work it seems certain that the second hypothesis is correct.

Summary.

Part I. The Course of Infection. Statistical Studies were made on twenty-three Birds infected with Proteosoma praecox.

1. Three types of infection were encountered, (1) primary acute infections, (2) extended irregular infections and (3) benign infections.

a. Four birds exhibited primary acute infections. Acute infections are short in duration, (5 to 10 days). Daily counts of the red cells and parasites showed an immediate decrease in red cells after inoculation; an inoculation period of from 12 to 23 days, followed by a rapid rise in the parasite count and a rapid fall in the red cell count; then the number of parasites decreased abruptly and the number of red cells began to increase a large percentage of them being normoblasts. (See tables 1, 2 and 3 and Figs. 1, 2 and 3).

b. Sixteen birds exhibited extended irregular infections. These infections are of long duration. Large fluct-

uations occur in the number of parasites present and whenever the parasites increase in number a corresponding decrease in redcells occurs. A gradual decrease in the number of red cells was noted during the incubation period and a continued increase in the number of red cells after a fall in the parasite number, as in birds with primary acute infections (See Tables 4, 5, 6 & 7 and Figs. 4, 5, 6 and 7).

c. Three birds exhibited benign infections. Such an infection is very mild and of short duration. Changes in the red cell and parasite counts were observed similar to those noted above but of a less pronounced nature. (See Tables 8 & 9, and Figs. 8 and 9).

2. Large mononuclear leucocytes increased in number as the number of parasites increased and decreased in number when a decrease in the number of parasites occurred. Since phagocytosis of parasites is known to take place in the blood, it is suggested that this process may be responsible in part for the decrease in the number of parasites present. (See p. 14 Plate III, Figs. 1-3).

Part II. Tissues of the spleen, bone marrow, brain, liver and kidney were examined from normal kinds, birds sacrificed at the height of the infection and at various times during the apyretic period.

Pathology.

1. Spleen. Two birds were sacrificed at the height of the infection. The changes in the spleen were (1) distention of endothelial cells, containing large quantities of pigment; (2) capillaries occluded with parasitized red cells; (3) an increase in the number of large mononuclear cells and mitotic figures; and (4) young parasites free or attached to mononuclear cells.

Sections of spleens of birds sacrificed at the end of the peripheral infection show a great decrease in the in the extent of the pathological changes described above.

Sections of spleens taken from birds at varying intervals during the latent infection show no perceptible alteration in the normal histology.

2. Marrow. The pathological changes in the bone marrow of highly parasitized birds are (1) a decrease in the adipose tissue and preponderance in mitotic figures and mononuclear cells; (2) a dilatation in the venous capillaries; (3) hyperplasia (4) parasitized red cells and free trophozoites.

At the end of the infection there is noticed a restoration of the adipose tissue and a decrease in mitotic figures, leucocytes, and normoblasts. Pigment and a small number of parasites were still to be seen.

No significant pathological changes were found in the bone marrow during the varying stages of the latent infection.

3. Parasites were found at the height of the infection in greatest numbers in the spleen and bone marrow and were less numerous in the brain, liver and kidney. Towards the end of the infections and during the apyretic periods they were only found in the spleen and bone marrow. At the end of the infections when it was no more possible to find parasites in the outer circulation organisms were demonstrable in the spleen and bone marrow. Two weeks, 4 weeks, 5 weeks, two and a half months and six months after the infections had disappeared from the blood a few parasites were found in the tissues after long patient search.

The parasites demonstrated were in various stages of development--trophozoites, schzonts and gametocytes. (See Plate II, III Figs. 1,2,3,4.5,6, & 2, 45)-and asexual reproduction was undoubtedly taking place.

Part III. Relapses occurred spontaneously in birds 9 & 14, and were induced artificially in birds 9, 14, 20 & 17.

1. Spontaneous relapses. A relapse occurred in Bird 9 one hundred and one days after the last parasite was found in the blood; gametocytes were present. Bird 14 relapsed forty-one days after the last parasite was found in the blood; all the parasites were schizonts.

2. Provocative relapses. From October 19 to Nov. 9 Bird 9 showed no parasites; upon radiation four schizonts were found. Fourteen days later another relapse occurred after an infection of a provocative dose of adrenalin; for a few days all stages of the organisms were found.

In Bird 14 an artificial relapse was induced. 37 days after its first and spontaneous relapse, schizonts, gametocytes and trophozoites were present. In Bird 20 a relapse was brought about 2 days after the end of the blood infection ; schizonts were found.

3. Studies of the Course of Infection, of tissue, smears and sections and of spontaneous and experimental relapses add convincing evidence to the hypothesis that during the afebrile periods of a malarial infection, the parasites are present in certain internal organs as well as in very small numbers in the blood, where they are continually undergoing asexual reproduction.

One factor that may prevent the actual increase in the number of parasites during the afebrile period is the phagocytosis of the young stages in the life cycle of the organism.

Tables 1 and 2.

Bird 9. Inoculated in breast muscle, May 31, 1922 with 0.5 c.c. of saline diluted blood from B. 8 containing large numbers of Proteosoma praecox.

Bird 19. Inoculated in breast muscle, May 14, 1922 with 0.5 c.c. of saline diluted blood from B. 14 containing fairly large numbers of Proteosoma praecox.

Bird No. 9 (1)			Bird No. 19 (2)		
Date injected	Blood per cmm.	Parasites per cmm.	Date injected	Blood per cmm.	Parasites per cmm.
May 31	4,270,000	--	Nov. 14	5,200,000	--
June 3	4,110,000	--	Nov. 21	4,800,000	--
June 15	3,990,000	--	Nov. 24	4,320,000	--
June 23	3,800,000	4,850	Nov. 26	4,300,000	1,900
June 24	2,880,000	17,325	Nov. 27	4,240,000	2,968
June 26	3,500,000	10,768	Nov. 28	3,900,000	10,140
June 27	4,000,000	2,199	Nov. 29	3,000,000	16,000
June 28	4,650,000	1,274	Dec. 1	3,161,000	11,060
June 29	4,850,000	one parasite	" 4	2,500,000	24,000
June 30	4,900,000	" "	" 5	2,500,000	28,000
Oct. 12	4,900,000	--			
Oct. 27	4,850,000	--			
Nov. 2	4,900,000	--			
Nov. 9	4,850,000	--			

Tables 3 and 4.

Bird 10. Inoculated in breast muscle, June 2, 1922, with 0.5 c.c. of saline diluted blood from B.

Bird 4. Inoculated intraperitoneally, April 23, 1922, with saline diluted blood from B 2 containing relatively few parasites.

Bird 10. (3)			Bird 4. (4)		
Date injected	Blood per cmm.	Parasites per cmm.	Date injected	Blood per cmm.	Parasites per cmm.
June 2	4,785,000	--	April 23	5,000,000	--
June 6	4,620,000	--	April 28	4,600,000	--
June 3	4,135,000	--	May 7	4,150,000	--
June 13	4,000,000	--	May 8	4,050,000	8,000
June 16	3,400,000	800	May 10	4,000,000	12,000
June 17	3,195,000	1,100	May 12	3,650,000	17,000
June 18	3,000,000	2,000	May 17	3,100,000	27,000
June 19	2,604,000	14,000	May 18	3,100,000	29,000
June 22	2,604,000	25,000	May 24	3,220,000	36,000
June 23	2,900,000	34,000	May 26	3,500,000	39,000
June 24	3,420,000	30,000	May 29	3,800,000	45,000
June 25	3,300,000	28,000	June 4	3,700,000	52,000
June 26	3,555,000	24,000	June 8	3,500,000	55,000
June 27	3,880,000	19,000	June 9	4,200,000	6,000
June 28	4,200,000	14,000	June 10	4,250,000	5,000
		Death	June 12	3,900,000	11,000
			June 13	3,430,000	15,000
			June 15	4,250,000	3,000
			June 17	3,800,000	34,000
			June 19	3,900,000	1,500
			June 21	4,200,000	4,000
			June 23	4,400,000	--
			June 24	5,200,000	--

Tables 5 and 6.

Bird 3. Inoculated intraperitoneally and in breast muscle June 22, 1922, with 0.5 c.c. saline diluted blood from B 2 containing relatively few parasites.

Bird 17. Inoculated in breast muscle, November 21, 1922, with 0.5 c.c. saline diluted blood from B 20 containing a fair number of parasites.

Bird 3. (5)			Bird 17. (6)		
Date injected	Blood count per cmm.	Parasites per cmm.	Date injected	Blood count per cmm.	Parasites per cmm.
June 21	5,000,000	--	Nov. 24	4,780,000	--
June 28	4,450,000	--	Nov. 29	4,600,000	--
July 1	4,050,000	--	Dec. 1	4,450,000	--
July 3	3,310,000	6,371	Dec. 3	4,400,000	--
July 4	2,900,000	15,657	Dec. 5	4,310,000	--
July 5	3,000,000	13,600	Dec. 8	4,160,000	--
July 7	3,500,000	5,500	Dec. 9	4,090,000	1,000
July 8	3,900,000	6,580	Dec. 10	3,920,000	2,400
July 10	4,070,000	14,820	Dec. 11	3,650,000	4,650
July 11	3,200,000	21,240	Dec. 12	3,060,000	5,650
July 12	3,060,000	31,500	Dec. 14	2,900,000	900
July 14	3,940,000	25,480	Dec. 15	2,600,000	2,000
July 15	3,730,000	26,560	Dec. 16	2,650,000	6,000
July 17	3,200,000	22,818	Dec. 17	2,750,000	12,000
July 18	3,010,000	52,910	Dec. 19	2,800,000	21,105
		Death	Dec. 21	2,900,000	11,000
			Dec. 22	3,120,000	13,700
			Dec. 23	4,000,000	9,000
			Dec. 24	4,150,000	7,000
			Dec. 25	4,400,000	5,000
			Dec. 26	4,600,000	2,600
			Dec. 27	4,820,000	0,600
			Dec. 30	5,100,000	--
			Dec. 31		--

Tables 7 and 8.

Bird 14. Inoculated in the breast muscle, October 18, 1922, in Dr. Whitmore's laboratory.

Bird 15. Inoculated in breast muscle, October 18, 1922, in Dr. Whitmore's laboratory.

Bird 14. (7)			Bird 15. (8)		
Date	Blood count per cmm.	Parasites per cmm.	Date	Blood count per cmm.	Parasites per cmm.
Oct. 20	5,200,000	--	Oct. 20	4,950,000	--
Oct. 30	5,120,000	--	Oct. 27	4,800,000	--
Nov. 1	5,000,000	--	Nov. 1	4,800,000	--
Nov. 2	4,900,000	2,064	Nov. 2	not made	--
Nov. 3	4,450,000	8,448	Nov. 3	4,660,000	1,296
Nov. 4	4,500,000	10,500	Nov. 4	4,550,000	4,200
Nov. 6	3,030,000	9,500	Nov. 6	4,000,000	3,000
Nov. 7	2,190,000	4,100	Nov. 7	4,470,000	1,000
Nov. 8	2,180,000	5,000	Nov. 8	4,750,000	--
Nov. 9	2,160,011	6,000	Nov. 11	5,030,000	--
Nov. 11	3,440,000	none made	Nov. 14	not made	--
Nov. 14	3,900,000	8,190	Nov. 29	5,120,000	--
Nov. 15	3,800,000	5,700	Dec. 9	5,420,000	--
Nov. 16	3,850,000	2,700	Dec. 11	5,400,000	--
Nov. 17	3,850,000	2,200	Dec. 18	5,500,000	--
Nov. 18	3,900,000	2,960			
Nov. 24	4,280,000	--			
Nov. 27	4,820,000	--			
Dec. 1	5,200,000	--			
Dec. 9	5,070,000	--			
Dec. 16	5,000,000	--			

Table 9.

Bird 20. Inoculated in breast muscle on November 12, 1922, with 0.5 c.c. saline diluted blood from B. 14 containing large numbers of Proteosoma praecox.

Bird 20. (9)		
Date injected	Blood count per cmm.	Parasites per cmm.
November 12	5,500,000	--
November 19	4,100,000	--
November 21	3,500,000	1,804
November 24	2,800,000	1,900
November 25	2,500,000	2,100
November 26	2,500,000	3,000
November 28	3,200,000	2,240
December 1	3,200,000	1,560
December 2	3,000,000	2,100
December 3	3,700,000	1,800
December 5	4,100,000	0,900
December 9	4,400,000	--
January 9	5,525,000	--

Table 10.

Tables of Birds 9 and 14 have already been presented. The following are to indicate the relapses of these birds.

Bird No. 9			Bird No. 14		
Date injected	Red blood cells per cmm.	Parasites per cmm.	Date injected	Red blood cells per cmm.	Parasites per cmm.
May 31	4,270,000	--	Oct.20	5,200,000	--
June 9	4,110,000	--	Oct.30	5,120,000	--
June 15	3,890,000	--	Nov. 1	5,000,000	--
June 23	3,800,000	4,850	Nov. 2	4,900,000	2,064
June 24	2,880,000	17,325	Nov. 3	4,450,000	8,448
June 26	3,500,000	10,768	Nov. 4	4,500,000	10,500
June 27	4,000,000	2,199	Nov. 6	3,030,000	9,500
June 28	4,650,000	1,274	Nov. 7	2,190,000	4,100
June 29	4,850,000	one parasite	Nov. 8	2,180,000	5,000
June 30	4,900,000	one parasite	Nov. 9	2,160,000	6,000
Oct. 12	4,900,000	--	Nov.11	3,440,000	none made
Oct. 27	4,850,000	--	Nov.14	3,900,000	8,190
Nov. 2	4,900,000	--	Nov.15	3,800,000	5,700
Nov. 9	4,350,000	Radiated	Nov.16	3,850,000	2,700
Nov. 12	4,740,000	3 schizonts	Nov.17	3,850,000	2,200
Nov. 17	4,400,000	2 schizonts	Nov.18	3,900,000	2,960
Dec. 8	4,800,000	15-3 hrs. search	Nov.24	4,280,000	--
Dec. 26	4,400,000	injected with adrenalin	Nov.27	4,820,000	--
Dec. 27	--	1 s. & 1 trop.	Dec. 1	5,200,000	--
Dec. 28	--	1. s.& 1 gam.	Dec. 9	5,070,000	--
Dec. 29	--	1 s.	Dec.16	5,000,000	--
			Dec.18	5,000,000	3,210
			Dec.20	4,000,000	1,600
			Dec.22	4,200,000	412
			Dec.28	5,130,000	--
			Dec.29		2,565
			Dec.30		2,565
			Jan. 5		1 parasite

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Biography.

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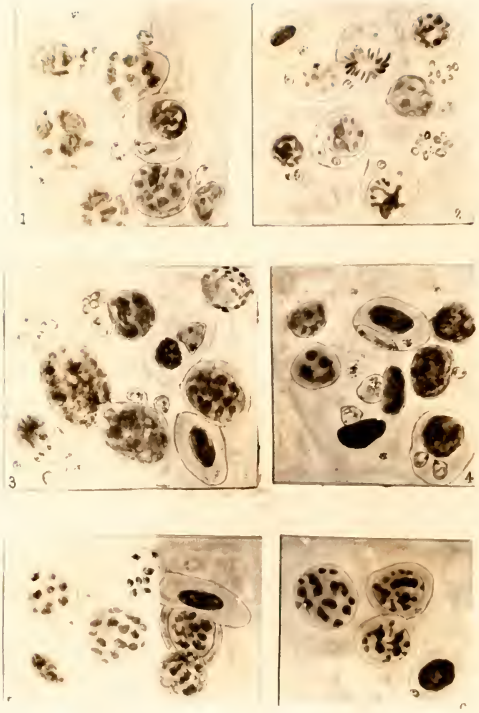
Degrees. A. B. from the Girls' College at Constantinop e

M. S. in Bacteriology from the University of Chicago.



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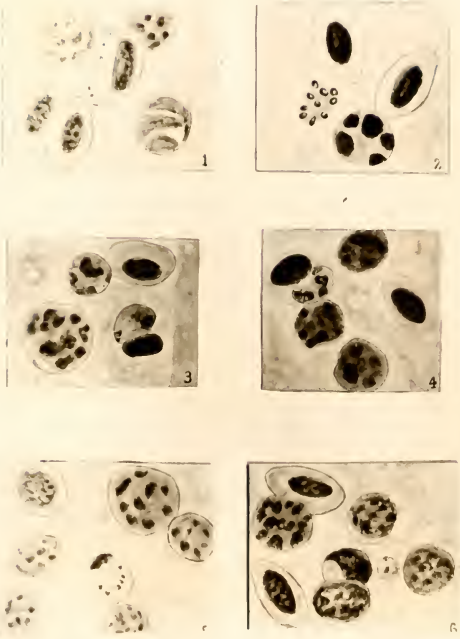

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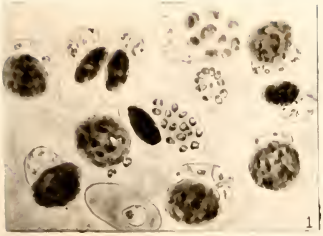
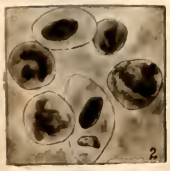
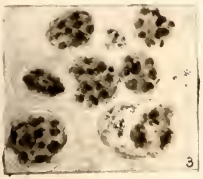
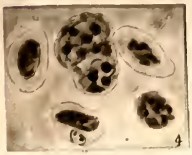
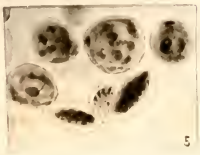










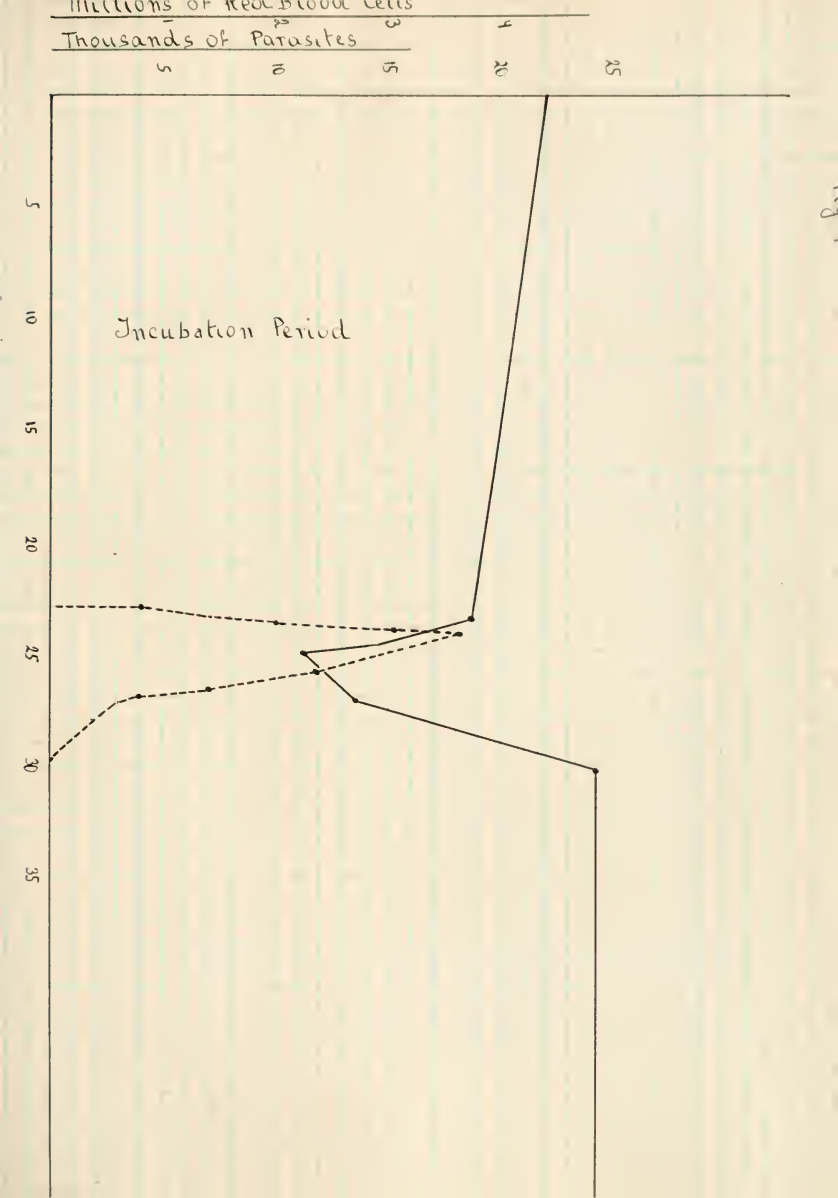

Millions of Red Blood Cells
Thousands of Parasites
5
10
15
20
25
Incubation Period

Fig. 2

30
25
20
15
10
5
Thousands of Parasites
Incubation Period
5
10
15
20
Days of Infection

FOLD OUT

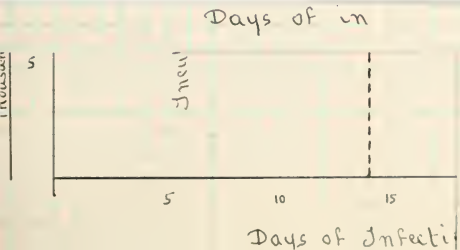


Fig 4

FOLD OUT

Millions of Red Blood Cells

FOLD OUT

Fig. 6

25
20
15
10
5
Thousands of Parasites
Incubation Period
5
10
15
20
25
30
35
40

millions of red blood cells

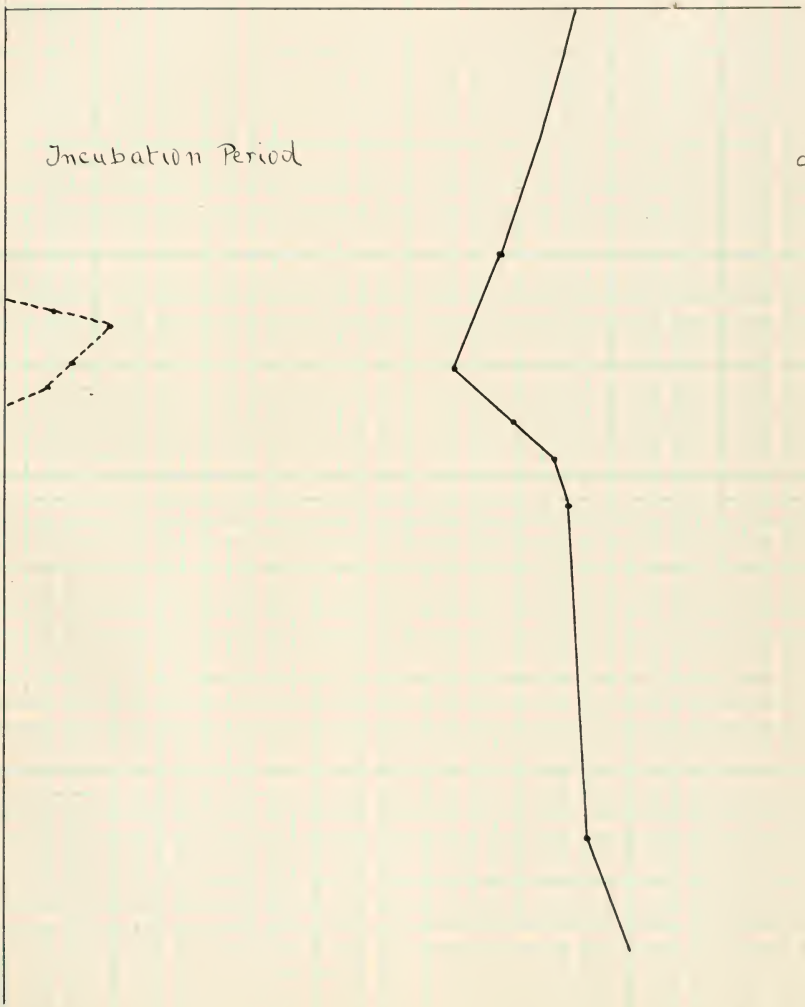
FOLD OUT

Millions of Red Blood Cells

1 2 3 4 5

Thousands of Parasites

5 10 15 20 25



Millions of red blood cells

Thousands of Parasites

Incubation Period

Days of Infection

Fig. 9

Millions of red blood cells
Thousands of Parasites
29 days
Adrenalin

P 45 Table 10